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Counting and measuring epibenthic organisms from digital photographs: A semiautomated approach

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Abstract

Benthic rocky bottom studies often investigate community structure and function where estimates of percentage cover and abundance of epibenthic organisms are required. Nondestructive photographic methods have the advantage of preserving benthic communities for repeated sampling. There is a need to accelerate image processing to make sample analysis more cost efficient and to make the data available in a timely manner. A semiautomated procedure to estimate epibenthic cover and abundance using Adobe Photoshop and the image analysis plug-in Fovea Pro was developed to meet this need. The method improves upon previous techniques by using color-based automated selection tools and a species-coding system. The technique required some manual processing because some species were less suitable for color recognition and the photographs were of inconsistent quality. The semiautomated selection of colony-forming organisms was validated by comparing it to a strictly manual approach using a data set from Balsfjord/northern Norway. Constrained ordination and Procrustes analyses showed that the automatic and manual methods were equally effective at documenting variation in the species/abundance data along the driving ecological gradient of depth. The minor deviations in species abundance estimation between the two methods (mostly <20%) were unrelated to the depth gradient and thus had negligible influence on the main ecological conclusions of the study. The semiautomated method is up to four times faster than the manual approach, has clear advantages over former benthic image analysis methods, and is well suited for detection of systematic biological patterns like ecological gradients.

Optical sampling techniques, such as underwater still photography and video recording, have seen rapid progress during the past three decades, providing a wealth of information that promises to reveal hidden facts marine scientists need to understand the systems they are studying (Solan et al. 2003). Recent developments and improvements in computer-based image analysis methods make these techniques available to a wider spectrum of study designs. The increasing volume of marine digital images calls for rapid and efficient processing methods to extract relevant, ecological information. The new technique presented in this article will help to increase efficiency (considerable time saving) in analysis of benthic hard-bottom photographs with no or minor loss in accuracy. We provide an example of a semiautomated image analysis using the software package Adobe Photoshop and the "Fovea Pro" plug-in (Russ 2001; Russ 2002).

Underwater photographical sampling has many advantages compared with conventional marine sampling methods: Photographs can be stored for practically unlimited time and can be re-examined whenever necessary. A much larger amount of data can be collected during the same period; a fact that is beneficial considering limited time and costs for marine field activities. An important feature is the nondestructive character of the method, which is a prerequisite to preserve specimens for subsequent taxonomic analysis (Baguley et al. 2004). It gives the scientist the opportunity to monitor the same sam-

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pling locations non-intrusively over time and eliminates confounding effects like trawl and grab sampling when investigating long-term changes in benthic habitats (Kollmann and Stachowitsch 2001). Benthic time-series studies using imaging techniques may focus on recruitment and succession (Beuchel and Gulliksen 2008; Pech et al. 2002; Stanwellsmith and Barnes 1997), megafaunal activity (Smith et al. 1993), climate variations (Beuchel et al. 2006), and population dynamics of selected species (Fujita and Ohta 1988; Tyler et al. 1993).

There are also important drawbacks of image-based sampling techniques. There are obvious limitations in quantitative accuracy of the obtained image data, and in reliable species identification. For this reason, photographs were, until recently, mostly regarded as precursor or complementary to conventional sampling techniques, or to make conventional survey techniques more effective and to improve the sampling design (Rumohr 1995). New advances in digital photography offer greater opportunities in underwater photography with the challenge being the development of more efficient ways for data extraction, analysis, storage, visualization, and processing (Solan et al. 2003).

Photographic techniques have already been used in a variety of benthic studies (e.g., Gutt et al. 1999; Gutt et al. 1996; Jørgensen and Gulliksen 2001; Pech et al. 2004; Piepenburg and Schmid 1997) where the main goal of image analysis was to extract information about covered areas or counting data of classified features in the picture. Volume and biomass estimations have also been conducted using photo images (Abdo et al. 2006; Baguley et al. 2004). Techniques involving color, edge contrast, or sharpness correction of the whole image or parts of it have been applied to improve data extraction and reduce processing time (Andresen 2003; Dahab et al. 2004; Taylor 2003b). Semiautomated and automated techniques have also been developed and improved to accelerate the analyzing process. The key here was to automate as many of the recurring tasks as possible, especially when large numbers of pictures should be analyzed (Taylor 2003a). One strategy was the automatic recognition of surfaces (Baguley et al. 2004) or landscape patterns (Teixido et al. 2002) that can be related to species or community characteristics. Another approach was the application of point-sampling methods by superimposing grids on the image and subsequent quantification of objects at the intersections of the gridlines (Gatlin et al. 1993; Roberts et al. 1994). Automatic separation of areas with the same or similar colors from neighboring areas, often referred to as color segmentation techniques, has also been attempted (Bernhardt and Griffing 2001; Borsotti et al. 1998; Gerald et al. 2001; Gerasimov 2000; Thornbush and Viles 2004).

Underwater photography is a useful, “nondestructive” method for obtaining information on conspicuous epifaunal organisms. Photographs of permanently marked areas over long time periods give the opportunity to study population dynamics (e.g., settlement, age, and mortality), individual growth and productivity, competition for space, predation, and community succession. We present a novel technique, which efficiently retrieves data on abundance and percentage cover from photographs of epibenthic organisms using Adobe PhotoShop CS (APS) and Fovea Pro. The described method has already been successfully applied in monitoring long-term changes in benthic community development on permanent sites at northern Norwegian and Svalbard coasts (Beuchel and Gulliksen 2008; Beuchel et al. 2006). Here we describe a way to optimize this method with a significant reduction in the amount of time needed for image analysis.

The purpose of the study is to compare two methods in selecting and extracting data of benthic organisms: one strict manual approach and one using automatic and semiautomatic selection tools based on color recognition. In our assessment, we test the two methods on photographs of colony-forming organisms, such as sponges and crustose algae, taken along a depth gradient. Our hypothesis is that the automated method would perform as well as the manual in detecting patterns of benthic community structure along an environmental gradient. Further, we address the question to which degree the automatic selection techniques work successfully across multiple images, and if the time savings are dependent on image quality.

Materials and procedures

Collection of photographs—The underwater photographs were collected by scuba diving, based on a technique developed by Tomas Lundälv (Lundälv 1971; Torlegard and Lundälv 1974).

The camera was a Hasselblad Super Wide Camera (SWC) with a Biogon 38 mm lens in a Hasselblad underwater casing fitted with a corrective glass port (including electronic flashes). The camera system was mounted onto a 0.5×0.5 m (0.25 m^2) metal frame enclosing the photographed bottom area to obtain quantitative data of abundance and percentage cover of bottom organisms.

Image processing—Conventional photographs based on positive reflectives or transparencies (slides), digital images, or digital still images extracted from video recordings using video capture equipment can be used for image analysis. In this study, slides were digitized using a flatbed scanner (Epson Perfection 3200 Photoscan) with a resolution of 1200 dpi. The obtained images were stored in TIFF format.

Measurements on the images were carried out in Adobe Photoshop CS (APS) and the commercial software extension of Fovea Pro v4.0 (Russ 2001; Russ 2002), using an Apple Power Mac G5. With the Fovea Pro plug-in installed, the interactive slide bar of APS filters is extended (Fig. 1) providing a variety of additional features. Combined with Photoshop Actions and Script capabilities, the plug-in can be used to automatically process folders containing batches of images, yielding data files ready for further interpretation in spreadsheet or statistics programs.

Before processing, the image format was converted to RGB (Red Green Blue), because most filters in Fovea Pro work only in RGB mode (Image → Mode → RGB Color). Then a copy of the background layer with the original image was made,

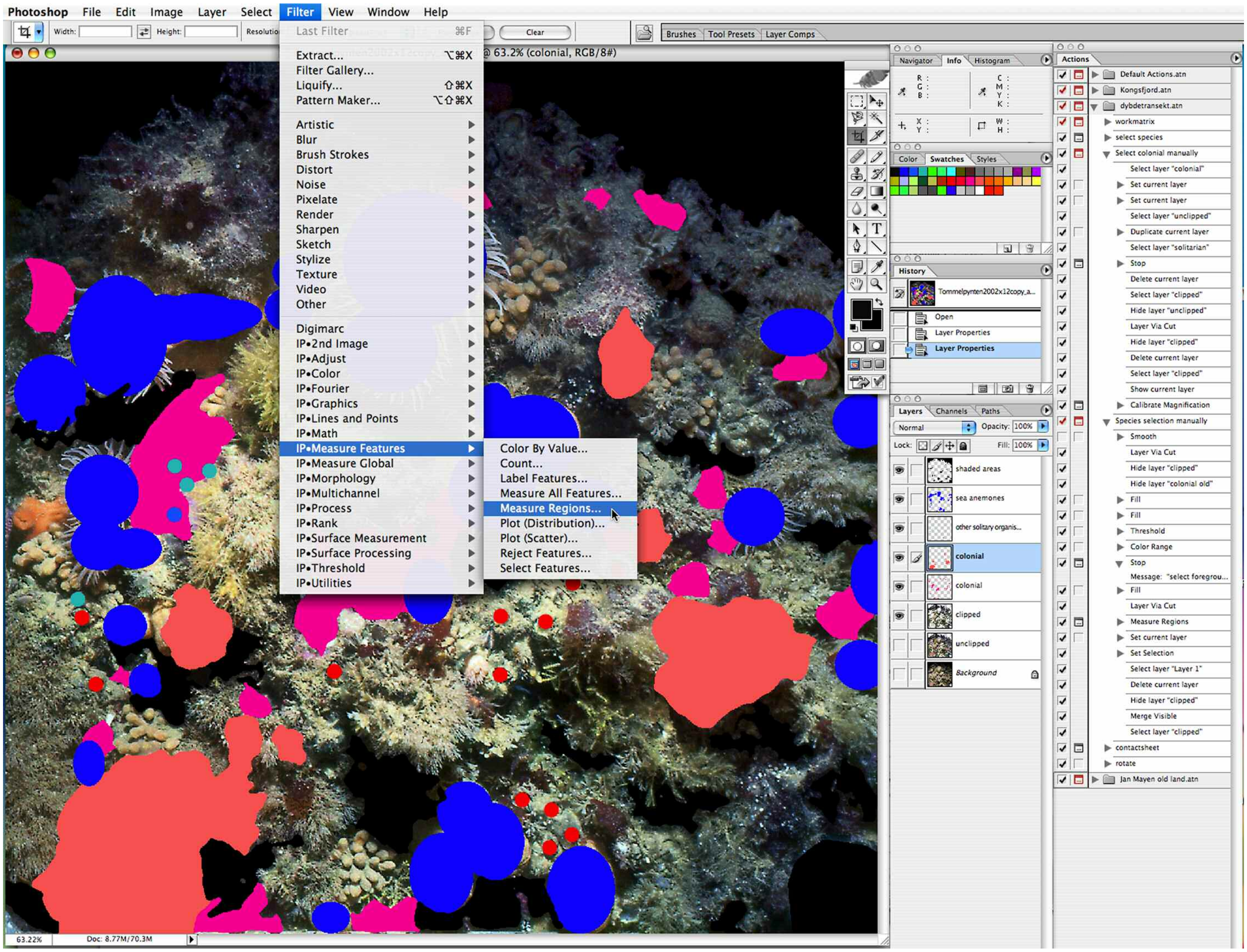

Fig. 1. Screen picture showing image analysis in Adobe Photoshop with the extended toolbar of Fovea Pro. Some areas covered by benthic organisms were already selected and filled with a color of a species-specific RGB code. Underexposed areas along edges and furrows are marked with black. Different layers were used for species selection, and repeating steps were executed automatically using Action files (window on the right border of screen).

which remained untouched during all further processing (Layer → Duplicate Layer) (Table 1). The next step was to create an image that contains the area inside the metal frame using the crop-tool from the tools palette (Fig. 1). Now, the image could be calibrated to its original size of 50 × 50cm (Filter → IP•Measure Global → Calibrate Magnification), so that all subsequent measurements referred to the size in situ.

Light and color distribution on the picture were adjusted to obtain consistent images suitable to apply the automated color sampling tools. Full automatic correction tools like "Auto levels" or "Auto color" yielded good results and were least time consuming (Image → Auto levels/Auto color). Due to the use of a flash, some areas were particularly over- or underexposed. Those areas were improved using the "Gradient tool" (tool palette) or the "Shadow/Highlight" filter (Image → Adjustments → Shadow/Highlight). Their use was more laborious than just the application of automatic tools.

There were many recurring steps during image processing and analysis (copying and deleting layers, color and light adjustments, assigning RGB codes to species, etc.). To automate and speed up the workflow, "action files" (Photoshop macros) were created using the Action palette. An action file is a series of commands that can be recorded at a certain time and "played" later. The macros contained breaks (pauses) that halt the automated process and allow for manual adjustment if needed (Fig. 1). During the initial stage of image processing, the same sequence of commands was batch processed using an "Action file" to increase time savings.

Image analysis—An essential step in the presented method was the assignment of a specific color-code to each species or

Table 1. Detailed instructions of Adobe Photoshop and Fovea Pro commands used in analysis of epibenthic organisms. The left column explains each step involved in measuring different types of organisms. The right column lists the commands to be executed and differentiates between menus, toolbars, dialog boxes, and literal instructions. (Legend: **Bold** = main menu; Underline = Toolbar, tools; *Italics* = Dialog box).

Steps and description	Commands in Adobe Photoshop and Fovea Pro
Image processing:	
Open image in Adobe Photoshop	**File** → Open → *select image file*
Convert color mode to RGB (most filters in Fovea Pro work only in RGB)	**Image** → Mode → RGB color
Select area of inner frame = area of interest, correct for distortion from camera	Tool bar → Crop tool (outline area of interest) → check box *Perspective* ☑ → fit crop area onto area of inner frame → press Return-button
Make a copy of layer with the area of interest, label new layer as "working layer"	Layer → Duplicate Layer → label with "*working layer*"
Adjustment of contrast and brightness	Image → Auto levels, auto color; or Image → Adjustments → Brightness/Contrast, Shadows/Highlight Tool bar → Gradient tool → Linear or radial gradient
Calibration of image, scale for measurements	Filter → IP•Measure Global → Calibrate magnification → Select *points* on upper right and left edges of image, set distance to known width of image (in our pictures 50 cm)
Selection of species and bottom features:	
Solitary organisms, small (only count data are desired): create new transparency layer for markers	**Layer** → New layer → label with "*small solitary organisms*" Tool bar → Brush tool, select diameter *~20 pixel*s → select species-specific color (previously defined) from Swatch bar → mark each with a dot.
Solitary organisms, small (in addition to count, also length or diameter data are desired):	Tool bar → line tool, select diameter *1 pixel* → select species-specific color (previously defined) from Swatch bar → mark each length or diameter with a line.
Solitary organisms, big (count and covered area data are desired) and colony-forming organisms that cannot be automatically selected: Manual selection of covered areas on "working layer," cut and transfer to a new layer	Choose Layers window → select "working layer" → Toolbar → choose Lasso tool or Magnetic lasso tool (when organism has a distinctive edge to neighboring features) from tool bar → encircle organism → **Layer** → Layer via cut → label with "*species name*"
Colony-forming organisms that can be selected semiautomatically: Automatic selection of covered areas on "working layer" by predefined or image-specific color range, smoothing selection to remove tiny areas, cut, and transfer to a new layer	Choose Layers window → select "working layer" → **Select** → Color range → Three alternatives: (1) *Load* (if pre-defined.axt file) (2) define desired color range by additive sampling using the *color picker* (+) (3) apply a combination of both, then → determine *fuzziness* (thresholds the color range) → *OK* → **Select** → Modify → Smooth *(3-5 pixels)* → *OK* → **Layer** → Layer via cut → label with "*species name*"
Shaded areas (due to use of flashlight, or in crevasses or furrows), sediments, or unpopulated areas	Same procedure as for selecting colony-forming organisms
Measurements:	
Fill selected area with species-specific color (color code in RGB)	Pick species-specific defined RGB color from Swatches bar, **Edit** → Fill → Use: *Foreground color*, Mode: *normal*, Opacity: *100%,* Preserve Transparency: *Yes* → *OK*
Measure selected areas of organisms	**Filter** → IP•Measure features → Measure regions
Reduce number of layers to keep file size small by combining layers for big, solitary, and all colony-forming organisms, respectively	Choose Layers window → Mark layers to be combined as 'visible' → Layers → Merge visible → label with, e.g., "*colony-forming organisms,*" repeat steps for other groups
Save file	**File** → Save as → *Select folder* and name the file

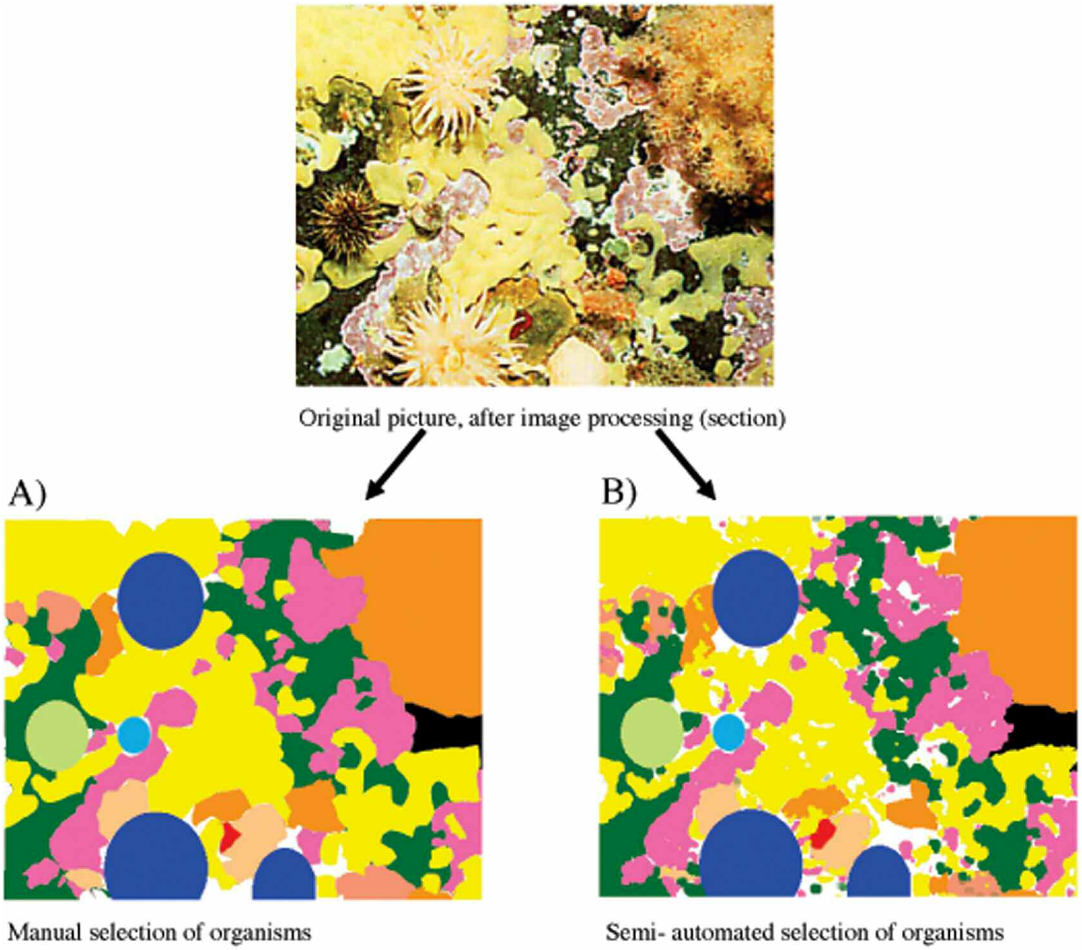

Fig. 2. Comparison of selection methods of colony forming benthic species from the same original picture using (A) the manual and (B) the semiautomated selection method.

taxon using the RGB model of APS. Each color channel of red, green, and blue encompasses values between 0 and 255 in the 8-bit color space, which results in a total number of 255^3 different colors. Before image analysis, a 9-digit color-code (containing the R, G, and B channel values) was created for each species or taxon and stored in a swatch-palette of Photoshop. First, the covered areas of a species in the image were selected, and then these areas were copied to a new layer and filled with the respective RGB-coding color using the macro procedure in APS. The color codes appeared along with the actual measurements in the results table and worked as identifiers for the respective species.

"Action files" were also used during image analysis, but unlike their use in image processing, no batch processing was applied. Each image was treated separately while running the "Action file." Recurring tasks were carried out automatically while the script stopped for manual work to be conducted.

During the analysis, we distinguished between solitary and colony-forming organisms. The relevant ecological data, like total abundance (counts) for solitary organisms and the area covered by colonial organisms (and large solitary species), were retrieved by applying different measuring instruments from the tools palette.

For the selection of solitary organisms with a radial form (e.g., actinarians, sea urchins, some solitary ascidians), the circle/ellipse marquees from the tools palette were used. Smaller motile organisms (e.g., chitons) or organisms with a very complex structure (e.g., sea stars) were marked with dots or straight lines. The measurements from such selections were the bases for analysis of, e.g., size distribution, age determination, and growth/production.

Two different methods were applied for the selection and quantification of colony-forming epifauna and macroalgae (Fig. 2). The methods differ in accuracy and efficiency and were compared in detail in the assessment study of this article. During the manual method, colonial taxa and bottom features were selected by surrounding their covered area using the "Lasso tool" and the "Polygonal lasso tool" from the tools palette. In addition, the "Magnetic lasso tool" was applied for faster selection of complex objects set against a high contrast

Table 2. Common groups of epibenthic organisms and the applied method of image analysis in Adobe Photoshop and Fovea Pro.

Species/taxon, bottom feature	Method*	RGB start values[†]	Tolerance level[†]	Degree of automation[‡]	Comments
Shaded areas	SA	0.0.0.	15–20	5	
Hildenbrandia sp.	SA	90.90.75	20–30	3	
Calcareous algae, *Lithothamnium* sp.	SA	230.210.200	20–30	4-5	Some manual touch up necessary because of variations in color
Calcareous algae, *Phymatholithon* sp.	SA	200.160.130	25–40	3	
Phæophycea	SA	100.100.70	20–30	4	Start values resemble *Hildenbrandia* sp., the areas of both species should be analyzed separately.
Porifera, colony-forming ascidians	SA	Start values differ due to large variations within same species	Dependent from inner-species variation	3	Some manual touch-up necessary because of variations in color
Hydrozoans, Bryozoans	SA	Start values differ due to large variations within group	Dependent from inner-species variation	1-2	Considerable manual work is necessary in most of cases
Gastropods, bivalves	M	N/A	N/A	0	Often complex structure or burrowers, outlined manually or marked with dots
Sea urchins, sea stars, brittle stars, sea cucumbers	M	N/A	N/A	0	Often complex structure, outlined manually, or marked with dots

*SA = semiautomated method, M = Manual method.

[†] RGB start values and the tolerance level refer to initial values of the color range selection tool in Adobe Photoshop.

[‡] Degree of automation: 0 = automation impossible, 1 = automation very limited, mostly manual outline, 2 = some automation, manual outline > 50%, 3 = automatic and manual selection approximately equal, 4 = high proportion of selection automated, minor manual touch-up, 5 = excellent automatic selection, occasionally manual touch-up.

background (Fig. 2). The selected areas of each taxon were then transferred to a new layer (Layer → Layer via cut) and filled with the assigned RGB-color for that feature (species or taxon).

The semiautomated method was applied to groups like sponges, ascidians, and calcareous algae that often showed an almost consistent surface and color (Table 2, Fig. 2). These characteristics made those taxons favorable for a color-based selection without tracing its outline (Select → Color range). The color-based selections were refined by additive and/or subtractive sampling and by limiting or expanding the color range. The color range of a specific taxon is defined by its RGB start and tolerance values that were stored in an "Action" file for use in multiple images. Another tool used for color-based selection was the "Magic wand tool," which works on similar principles as "Color range." In most cases, the full automatic "Color range" and "Magic wand tool" needed some manual touch-up to obtain satisfying selections.

Areas not inhabited by any organisms were also measured to quantify the entire area within the frame. Such areas could be sediment deposits or bare rock. Solitary organisms that had more than half of their body surface outside the frame were also measured, but later omitted from the total area.

In some cases, the quality of the underexposed parts of the image could not be improved and thus no useful information could be retrieved. Those areas were quantified as "shaded areas" and subtracted from the total area of the image. They were often found along the image borders due to shading effects from the metal frame. Deeper furrows in the bedrock posed a similar challenge as well as large stones and organisms (Fig. 1). Shaded areas were successfully extracted using color range functions (Table 2)

For the calculation of abundances, we used different community layers to correct for errors due to the top-down view of the camera system. To simplify the calculation, we distinguished between three community layers: (1) large, erect brown algae, (2) large, solitary organisms like sea anemones and sea urchins, and (3) all small solitary organisms and encrusting colonial organisms close to the rock surface.

The output data were in simple text format and could easily be imported into a spreadsheet. Each row in the file consisted of data from a single measurement (a solitary organism, a single colony, or an abiotic feature). The data file contains parameters such as diameter, the covered area, the perimeter, and the position (coordinates) of an organism within the square. The semiautomated color sampling resulted in a large number of very small areas of only few pixels. Such areas were efficiently removed by smoothing the selection (Select → Modify → smooth → 3-5 pixels).

Assessment

Photographs for this study are from a near vertical transect along a steep rock wall that extends from the surface down to about 100 m at Haugbergnes, Balsfjord, northern Norway (69°31′09′(N; 19°00′30′(E). Photographs were taken in October 1991 from 2 to 44 m depth, in depth intervals of 2 m. For this assessment, we selected 10 pictures from different depths along the transect with a high amount of colony-forming organisms to compare the two different methods of image analysis.

Statistical analysis—To compare the information obtained by manual versus automatic image-processing techniques, we used ordination methods followed by Procrustean analysis, so as to assess the similarity between the two data sets (Legendre and Legendre 1998; Peres-Neto and Jackson 2001). We first summarized the benthos data by indirect ordination (Correspondence Analysis [CA]) on the combined manual and automated data, to inspect the deviations between paired (manual and automated) observations in the reduced ordination space represented by a biplot (Greenacre 2007). Second, we performed two separate CAs, one for each data set, to obtain ordination matrices that could be compared by Procrustean analysis. To obtain a measure of discrepancy between ordination results, we applied a Procrustean superimposition (Peres-Neto and Jackson 2001). The measure of discrepancy could then be tested for randomness by permutation. The same sequence of ordination and Procrustean analysis was thereafter performed using Canonical (Constrained) Correspondence Analysis (CCA), a direct ordination technique (Greenacre 2007; Ter Braak 1987). Constrained ordination was first applied on the combined data set, with depth and analyzing method (including their interaction) as explanatory variables. Thereafter, separate CCAs were applied to the two data sets, using depth as explanatory variable, followed by Procrustean analysis of the constrained ordination results. The rationale behind an analysis of discrepancy between data sets in the constrained ordination space is that by focusing on the main ecological gradients, we could identify deviations between data sets that are relevant for ecological interpretation.

Spatial distribution—In the assessment of our semiautomated method, 11 different colony-forming benthic taxa were detected and analyzed. In addition, areas that were shaded due to use of flash were also estimated using both semiautomated and manual method (Fig. 3).

The two taxa of bottom-covering algae (*Hildenbrandia* sp. and calcareous red algae) were mostly found in the upper depths. Calcareous red algae showed a continuously decreasing abundance from 14 m (covering ≈50% of the total area) to 34 m (<5%). Five different taxa of sponges were detected, of which two could be identified to genus level. They occurred mostly in middle and lower depths. *Halichondria* sp. showed highest densities at depths between 24 and 32 m, covering up to 10% of the total area. Porifera indet.2 was the most abundant sponge, covering up to 30% of the photographs at depths between 34 and 44 m. Bryozoans were found across the entire depth transect, with increasing abundances below 28 m. Two taxa of ascidians (*Botryllus* sp. and *Didemnum* sp.) were found at the lowermost depth (44 m) and occurred in low densities.

Differences between manual and semiautomated method—There were no systematic differences in estimation of covered area between the manual and the semiautomated method (Fig. 3). The deviations were both positive and negative for most of the taxa at different depths. Calcareous algae, however, tended to be underestimated (maximum 22% at 24 m) by the automated method, whereas bryozoans and ascidians were often overestimated (highest values 26% for bryozoans at 34 m and 17% for *Didemnum* sp. at 44 m). The differences between the manual and semiautomated method were in most cases < 20% (in 78.7% of measurements, n = 47 and >30% in only 2.13% of all measurements [Fig. 4]).

The deviations seen in the CA between the manual and semiautomated method were very low. Axis CA1 accounted for about 55% of the observed variation and showed clearly a gradient related to depth. Axis CA2 accounted for 18% of the observed variation (Table 3). In the CCA, the inertia of the constrained axes (0.498) made up almost half of the total inertia of all axes (1.094, Table 3). The main gradient (CCA1) was very closely associated with depth (Fig. 5) where most of the species and depth intervals were grouped along this axis. Species associated with upper depths (euphotic zone) were *Hildenbrandia* sp., calcareous red algae, and Bryozoans 2, whereas different types of sponge and the ascidia *Didemnum* sp. were more related to lower depths. The inertia of the depth gradient (0.494) accounted for almost all the constrained variation in the benthic community, whereas the differences between the manual and semiautomated methods that are close related to CCA2 (inertia = 0.003) were negligible (Table 3). In the Procrustes analysis, the Procrustes errors based on CCA are low (0.164) and the correlation factor between the two Procrustes matrices is very high (0.993) (Table 3). The deviations between manually and semiautomatically processed pictures are predominantly orthogonal to CCA1 and the depth gradient (Fig. 6).

Discussion

The benthic community at Haugbergnes is clearly structured along a depth gradient. Almost all variation in the CCA was related to this axis (Table 3, Fig. 5). In close vicinity to our location, a strong depth gradient structuring the benthic community was found by Jørgensen (2001) when a similar range of depths was investigated using a diver-operated suction sampler. Comparable with our results, algae dominated at 10 m depth, whereas sponges and bryozoans were more abundant

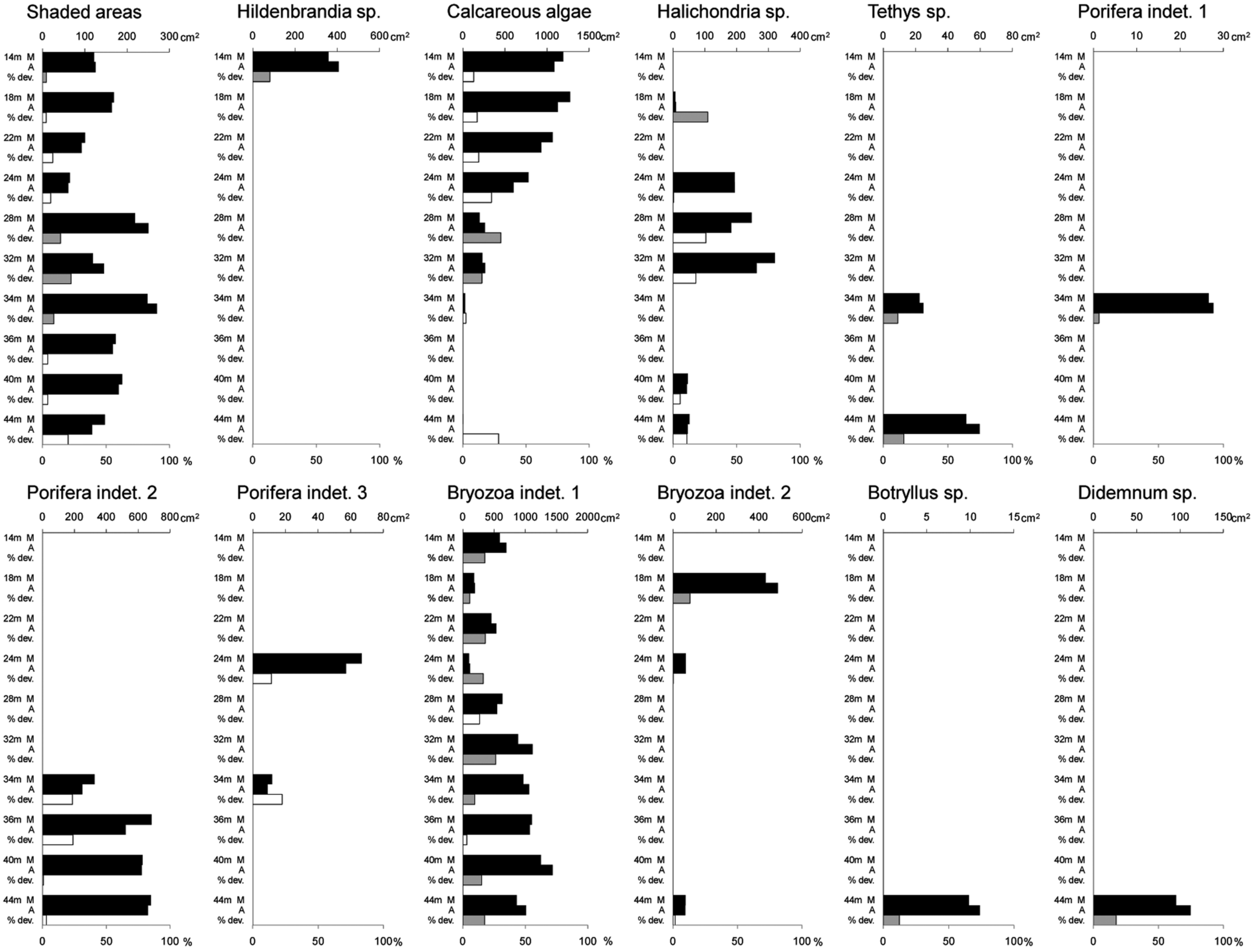

Fig. 3. Species distribution of benthic taxa at Haugbergnes. Black bars indicate the covered area (upper *x* axis) for each species at different depths, obtained by the manual (M) and semiautomated (A) method. Percentage deviations between the two methods are given at each depth (lower *x* axis) as gray bars (if positive deviation) and white bars (if negative deviation).

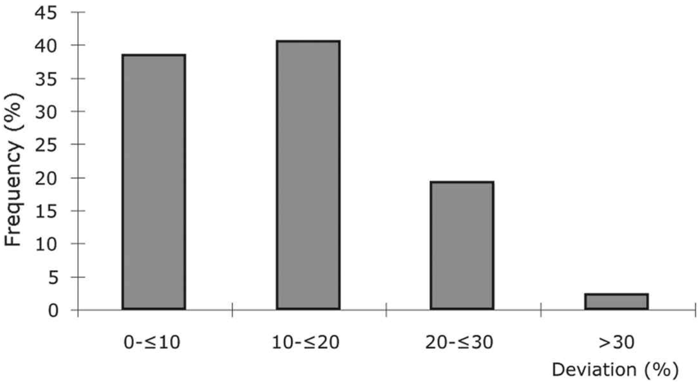

Fig. 4. Frequency of relative deviation between manual and semiautomated treated pictures of cover of selected benthic taxa at Haugbergnes.

at 20 and 30 m depths. Filter-feeding rocky bottom taxa such as poriferans, hydrozoans, bryozoans, and tunicates were associated with vertical and overhanging sites. These taxa probably benefit from limited sediment action, which would clog their feeding apparatus (Evans et al. 1980). Algae are poor competitors with increasing depth due to the reduced amount of light, which opens more space for animal settling (Sandnes and Gulliksen 1980). Vertical walls are usually less favorable for predators like sea urchins, so the community appears to be structured mainly by competition for space among the organisms (Sebens 1985).

The CCA showed that the differences in species abundance estimation between manual and semiautomated processed pictures were negligible and unrelated (orthogonal) to the driving ecological gradient of depth (Fig. 5). Procrustes analy-

Table 3. Results of ordination and procrustes analysis for the benthic community at Haugbergnes.

Analysis	Total inertia	Inertia-unconstrained axis	Inertia-constrained axis	Eigenvalues Axis 1	Eigenvalues Axis 2
CA	1.094	1.094		0.553	0.187
CCA	1.094	0.596	0.498	0.494*	0.003*

	Sum of squares	Root mean squared error	Correlation in symmetric Procrustes rotation	Significance
Procrustes analyses based on CCA	0.268	0.164	0.993	<0.001†

*Constrained axis

†Based on 1000 permutations

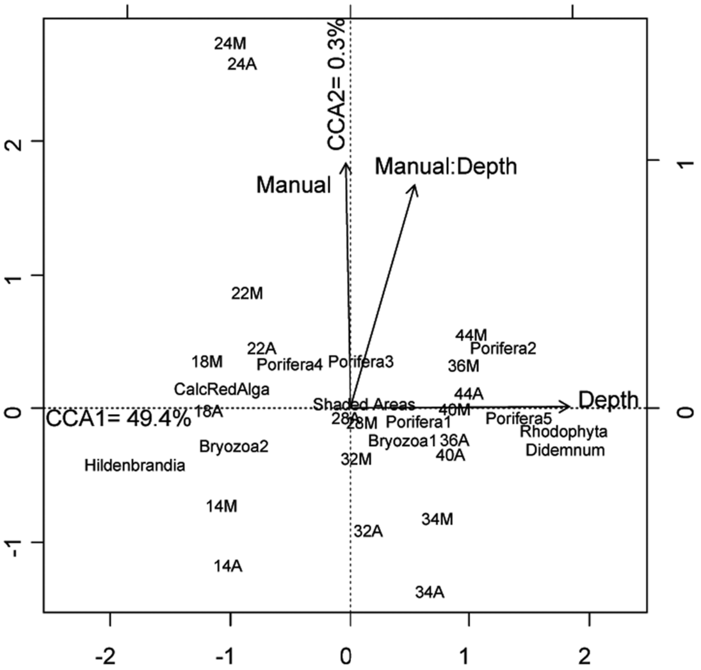

Fig. 5. Triplot of CCA results, including taxa, samples, and constrained factors depth and method (Manual).

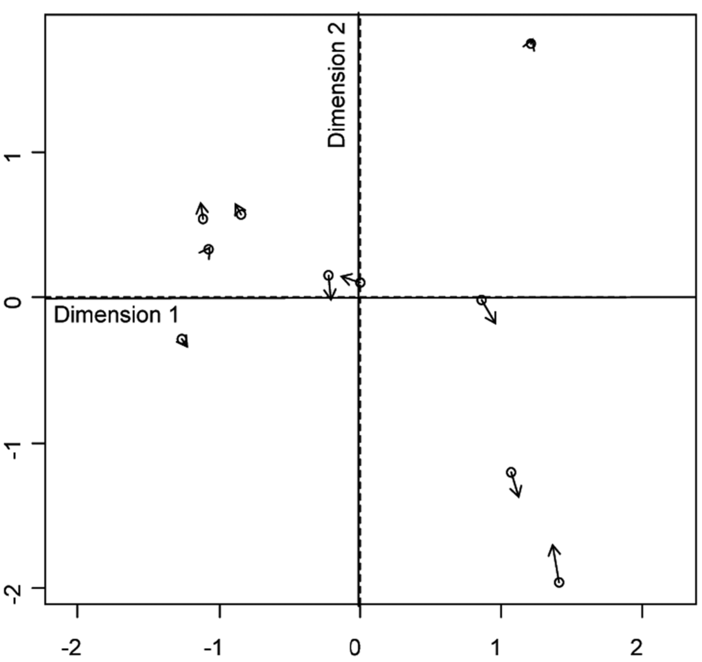

Fig. 6. Procrustes analysis based on CCA results showing deviations between manual and semiautomated methods.

ses confirmed this pattern showing that the paired deviations between the two methods were also predominantly orthogonal to the depth gradient. They, therefore, did not influence the main ecological result (i.e., the importance of the depth gradient), and would thereby not constitute a serious limitation of the automated method. Thus, the main conclusions about an environmental gradient structuring the benthic community was not affected from differences between the two methods, whereas the amount of time needed for processing and analyzing the images decreased considerably with the semiautomated method.

Comments and recommendations

Many techniques in estimating cover and abundance of epibenthic organisms based on imaging techniques (e.g., Rumohr 1995) are described as elaborate and time-consuming. Our new approach using APS and Fovea Pro is an improvement over previous methods. It significantly reduces the former efforts needed to manually outline and measure complex structures of rocky bottom ecosystems of combining semiautomatic and manual techniques. The method meets the challenge in combining efficiency with the accuracy that is required in modern ecosystem analysis. We suggest that APS and Fovea Pro provide a valuable alternative to expensive custom-made image analysis systems for many applications in benthic-ecological investigations.

The semiautomated estimates of cover of colonial benthic organisms and macroalgae are not unreasonably different from direct measurements, since differences between those estimates were mostly below 20%, with deviations randomly distributed in both positive and negative directions (Fig. 3). However, there were considerable time savings applying the new method: The amount of time used for image processing and analysis decreased from approximately 1 h using the manual approach to between 15 and 30 min (based on image quality and amount of organisms that could be extracted automatically). This corresponds to 50% to 75% time saving for a skilled person. These estimates were derived from this, as well as earlier studies where the same technique was used (*see* Beuchel and Gulliksen 2008; Beuchel et al. 2006). The new approach of using automatic processes of color segmentation for a number of colony-forming species yielded good results (Table 2). The method was excellent when applied to species with distinct and consistent colors, like calcareous red algae (e.g., *Lithothamnium* sp.), brown algae, and some species of sponges and colony-forming ascidians (e.g., *Didemnum* sp.). It also worked sufficiently on bryozoans and hydrozoans, but the pre-processing of the images including adjustments of illumination, color, and contrast was assessed to be essential to improve the accuracy of semiautomatic color segmentation (Bernhardt and Griffing 2001). Another benefit of our method is the permanent and easily accessible photographic record of each bottom organism or feature. Those are cross-referenced to data sheets via the RGB coding system, and thus, offer the possibility for later re-examination. Furthermore, the technique is nondestructive, which provides great opportunities for monitoring the same bottom locations over long time without physically changing the benthic communities. The semiautomated method can also be applied to other benthic habitats to estimate cover and abundance, especially when there is a distinctive difference in color and structure between the taxa. A vast proportion of the benthic imagery is collected with video. We have tried to apply our technique to video imagery, with some promising results (no data available). Higher resolution (high definition) and slower movement of the camera system yielded better results, but the outcomes are still below the quality of still images. Since 2005, the same technique has been applied to digital still pictures, with a doubling of the resolution compared with the presented method based on scanned slides. Much smaller individuals could now be recognized, and species determination has been improved.

There have been some drawbacks of the semiautomated method that can be improved in the future. The sampling based on color range worked well in uniformly exposed and high contrast photographs, but became more labor-intensive in pictures with severe shaded or underexposed areas. In addition, color and morphological variation within a species could be large at small spatial scales. Those pictures needed additional "touch-up" to the automated technique, using manual tools (e.g., the lasso tool or magic wand tool) to increase accuracy. Many species of sponge and ascidians could not be reliably identified from photography. We recommend taking physical samples of epibenthic fauna from nearby locations for identification in laboratory to overcome this problem, but some uncertainty will always persist. In many cases, identification from pictures must be made at a higher taxonomic level.

In the future, a key issue will be the improvement of photographic techniques to increase the quality of the initial image and to save time for image processing. It seems that the skilled use of a flash has the potential for more equally exposed photographs. Some tools to alter the exposure of the whole or parts of the image were applied during the image processing in APS, but those adjustments are limited and cannot replace an original photograph of better quality. We tried, e.g., the "gradient tool," which delivered good results, but its application is often tedious and time-consuming. Another suggestion was to photograph the quadrates with standard color tiles or black and white standards, as this would help in the initial color correction and reduce image-processing time. We used only few of the available filters of APS and Fovea Pro in our study, but there are many possibilities for further development of the method using these tools. The aim should be to increase the amount of automatic recognition including more species, but due to the complexity of epibenthic hard bottom substrates, it is hard to believe that fully automatic species recognition will ever be achieved.

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